QAPP

Shipboard test of ship's BWMS

Prepared for:Jiujiang Precison Measuring Technology Research Institute

Prepared by:Ballast Water Detecting Lab of Shanghai Ocean University

April 2013

A. project management

Quality Assurance Statement

To assure the quality and rationality of the laboratory tests, we hereby declare that the shipboard test and the sampling procedure of the ballast water management system conducted by us are strictly in accordance with the requirements of this Quality Assurance Project Plan, the Guidelines for Approval of Ballast Water Management Systems (G8) and the Guidelines for Ballast Water Sampling (G2).

Term of validity:

Term of validity of the QAPP; 1th April 2013 to 1th April 2014

Project supervisor: Li Shulin Research professor

Technical director: Zhang Daiyi Research professor

Quality manager: Sun Anxin Engineer

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Quality Assurance Statement

To assure the quality and rationality of the laboratory tests, we hereby declare that the sampling and testing procedures conducted by us are strictly in accordance with this Quality Assurance Project Plan, the ISO/IEC 17025: 2005, the Guidelines for Approval of Ballast Water Management Systems (G8) and the Guidelines for Ballast Water Sampling (G2).

Term of validity:

Term of validity of the QAPP; 1th April 2013 to 1th April 2014

Project manager: Xue Junzeng Professor

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Note: the quality assurance project supervisor of the test of ballast water management system will keep this file as a project quality assurance record and a basis for the following test.

A2. content

A2.1 Content of table

A2.2 Content of figure

A3. Brief Introduction of Participating Organizations

A3.1 Ballast Water Detecting Lab of Shanghai Ocean University

The Ballast Water Detecting Lab of Shanghai Ocean University was founded in September 2008. There are eighteen people in the lab, among which four people are engineers with high professional title. The lab consists of sample acceptance room, hydrochemistry room, micro organism testing room, microscope room, sample storage room. The lab is dedicated to the study of the harbor ecology, mainly of the ecology research study of the plankton in harbor area and ship ballast water, and the study of micro organisms' ecology in ocean environment. This organization has published over 100 papers in both national and international academic journals. In addition, the lab has obtained 20 patents authorizations.

The lab is equipped with all kinds of instruments and apparatus, such as BOD_5 analyzer, TOC analyzer, spectrophotometer, stereoscopic microscope, conductivity gauge, turbidimeter for water micro-organism test, environmental parameters detection and plankton test. The related staff is required to be trained before he or she can perform the testing. The six doctors and twelve masters are all specialized in the field of the tested parameters. By now, the lab is able to test five indicator microbes and ten water quality parameters in accordance with the ballast water discharging

standards regulated in the International Convention for the Control and Management of the Ships' Ballast Water and Sediments:(1) viable organisms greater than or equal to 50μm in minimum dimension; (2)viable organisms less than 50μm and greater than 10μm in minimum dimension; (3) toxicogenic *Vibrio cholerae*(serotypes O1 and O139); (4)*Escherichia coli*; (5)*Intestinal Enterococci* ;(6) heterotrophic bacteria;(7)total residual oxidants (TRO); (8) dissolved oxygen(DO); (9) total suspended solids(TSS) ;(10) turbidity (NTU); (11) dissolved organic carbon (DOC) ; (12) particulate organic carbon (POC) ; (13) pH; (14) salinity; (15) water temperature.

Being realistic and creative, the staff of the lab aims to build a competent and famous lab which is specialized in the testing of ships' ballast water in China.

A3.2 Jiujiang Precision Measuring Technology Research Institute

Jiujiang Precision Measuring Technology Research Institute is a comprehensive research organization which is engaged in precision test, precision processing, and precision measuring. It is a subsidiary company of CSSC (China State Shipbuilding Corporation).It is located in the economic development zone of Jiujiang City. Now, the institute has a total floor area of 92, 800 square meters, building area of 30,000 square meters, and total assets of more than 226 million Yuan (RMB). It also owns more than1,600 sets of equipments including metering, measuring, processing apparatus and also computers. And it also is equipped with a lot of advanced mechanical and electronic computer aided design software, analysis software and simulation software.

OceanDoctor BWMS is developed and manufactured by Jiujiang Precision Measuring Technology Research Institute for ballast water treatment. The design and test of the system are conducted strictly in conformance with the IMO's Guidelines for approval of ballast water management systems (G8), Res. MEPC.174(58) and Procedure for approval of ballast water management systems that make use of active substances (G9) MEPC.169 (57), it is verified by the performance test that the

performance specifications are consistent with the D-2 discharge standard stipulated in International Convention for the Control and Management of Ships' Ballast Water and Sediment.

A4. Project Organizational Chart

Research and development organization:

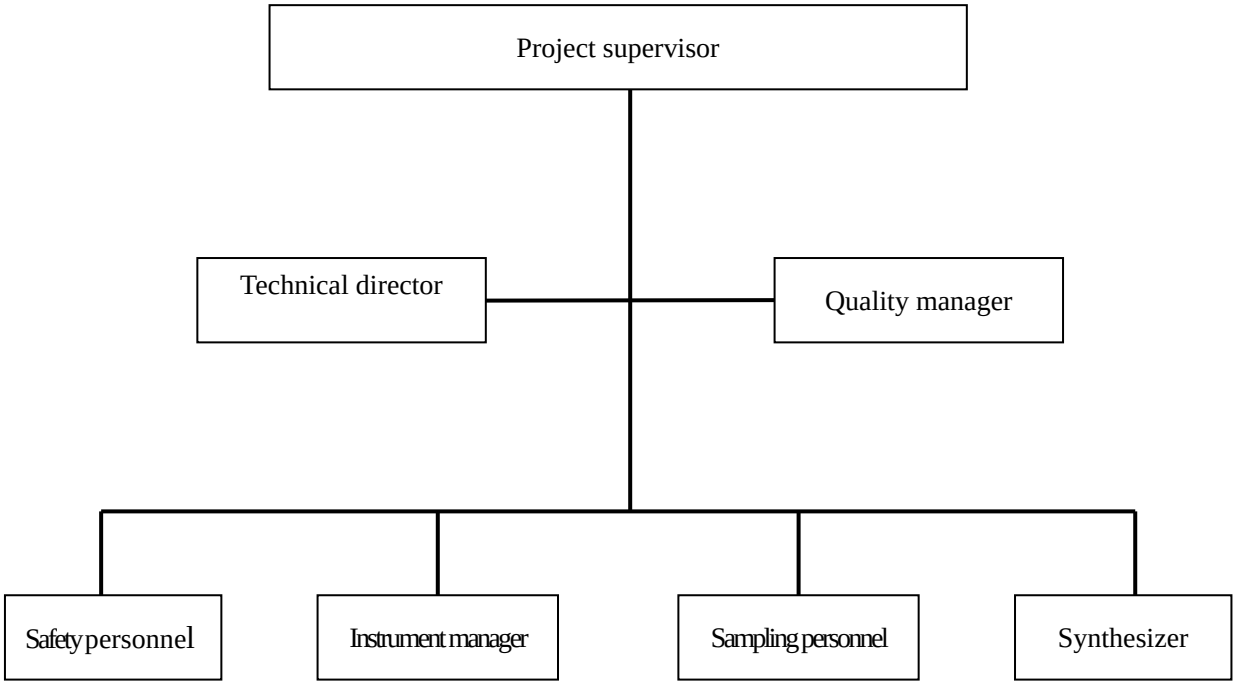

Figure 1 Organizational Chart of the Research and Development Organization

Project supervisor: Li Shulin

The project supervisor is responsible for the overall management work of the project. It is the responsibility of him to organize the human resource; material resource and financial resource of the company to ensure that the project goes on wheels. He is responsible for establishing the quality policy and quality objectives and arranging the work schedule of the project, and also he has the responsibility for urging the staff of the project to follow the requirements of management system files and regulations of the company.

Technical director:Zhang Daiyi

The director is responsible for the overall technical works of the project, and he is responsible for the technical training of the personnel involved in the related test work, arranging the experimental flow and technical principle studying for the related staff. Organizing and coordinating the development of the test is also one of his responsibilities. He is also in charge of dealing with the emergencies occurred during the test process. Moreover, he is responsible for the assurance of test tempo and device status to be in compliance with the requirements of the QAPP.

Quality manager:Sun Anxin

He is responsible for the quality related work and ensures that the quality objectives to be fulfilled. In addition, he is in charge of the safety, healthy and environmental protection work throughout the development of the project. He is responsible for supervising the staff of the project team to finish the work in accordance with the QAPP.

Safety personnel: Zhao Jiliang

He is responsible for the safety of the test field. Keep eye on the safety of the test field, give suggestions on how to deal with the potential safety hazard and monitor the implementation of the improvement measures and ensure that the test run in order.

Instrument manager: Ma ji

The operation of the ballast water management system in compliance with the requirements in the test process is one of his responsibilities. And keep record of it. Report to the technical director about the running status of instruments and help the technical director with solving the defaults of the instruments.

Synthesizer: Liu Gang

He is responsible for the management of the document and files over the whole course of the project. Organize and store the documents according to the requirements. He is also in charge of the coordination of the resources in the field test.

Sampling personnel: Zhou Yue

He is responsible for assisting the test organizations in collecting the samples .Ensure that the samples are classified and managed in order.

Project test organization:

Organizational Chart:

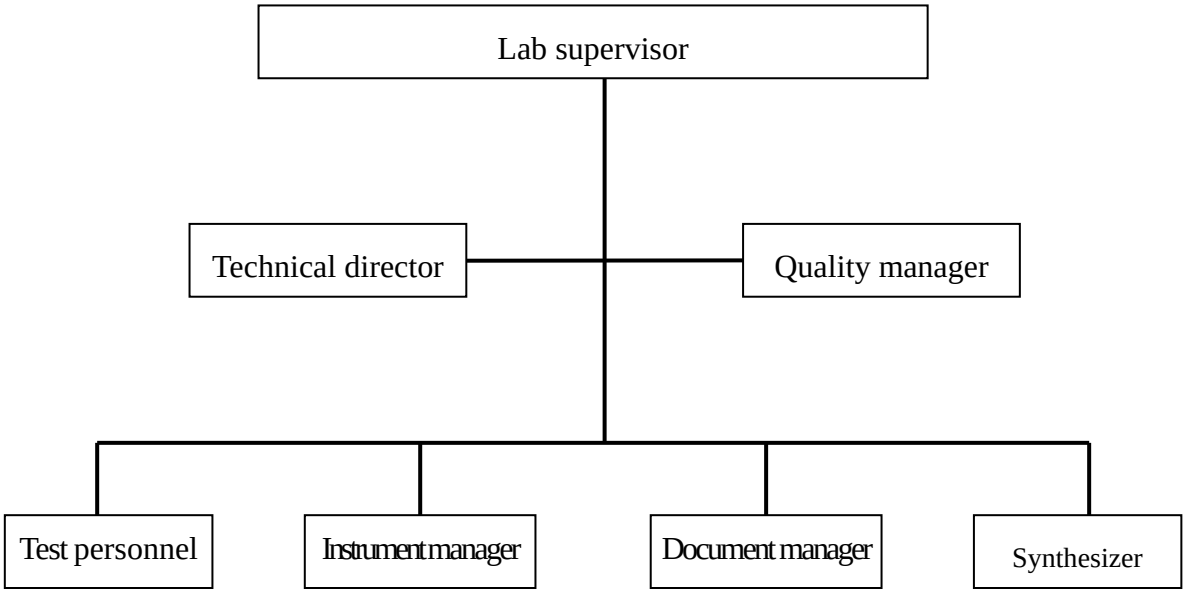

Figure 2 Organizational Chart of the Test Organization

Lab supervisor;Xue Junzeng

He is responsible for the overall project management work and communicating with the research and development organization. He is responsible for the organization structuring and resources allocation. And he is also responsible for establishing the quality policy and quality objectives and arranging the work schedule of the project, and also he has the responsibility of urging the staff of the project to follow the requirements of management system files and regulations of the lab.

Lab director /technical director: Wu Huixian

She is responsible for the overall technical works of the project. Also, she is responsible for the training of the testing personnel and organizing the related staff for test result analyzing; she will supervise and also help the testing personnel to finish the test in accordance with the related requirements and solve the technical problems that may arise in the work process; she is also responsible for making the plan for developing the project and organize the implementation of the plan; moreover, she is asked to ensure the validity of the test standard in use.

Quality manager: Wang Qiong

She is responsible for the quality assurance work for the project and ensures the achievement of the quality objectives. She is also responsible for the custom acceptance and dealing with the custom complaints. In addition, she is responsible for the safety, healthy and environmental protection work during the project development. And she also plays the role of lab internal affairs supervisor.

Testing personnel: Wang Qiong, Yuan Lin, Bian Jiayin, Liu Liang, Liu Yan, Yu Jiafeng and so on.

Liu liang and Wang Qiong as the testing personnel of the project are mainly in charge of testing of viable organisms greater than or equal to 10 micrometers and less than 50 micrometers in minimum dimension. Xue Junzeng and Wang Qiong are responsible for the testing review of the viable organisms greater than or equal to 10 micrometers and less than 50 micrometers in minimum dimension. Wu Huixian and Liu Liang are in charge of the review of the bacteria testing .Yuan Lin is responsible for the testing of the viable organisms greater than or equal to 50 micrometers or more in minimum dimension; Liu Yan and Bian Jiayin are responsible for the testing review of the viable organisms greater than or equal to 50 micrometers or more in minimum dimension. Bian Jiayin is in charge of the testing of the environmental parameters. Liu Liang and Yu Jiafeng are in charge of the testing review of the environmental parameters. In addition, the technical director is responsible for the field sample collection. The sampling personnel are Wang Qiong, Yuan Lin, Wu Huixian, Liu Liang and so on.

The testing staff is asked to conduct the testing work carefully to assure the validity of the testing data. Make sure that the environmental condition of the lab meets the requirements and keep the lab tidy and safety. The staff is required to participate in the training to enhance their awareness of the importance of the quality and the testing ability. The review personnel should master the methods for parameter testing and the uncertainty of the testing result, and review the initial data and testing results objectively and scientifically. All the testing personnel and the review personnel should be responsible for keeping the technical and commercial secrets of the

custom.

Field sampling personnel must carry out the sampling work strictly in compliance with the sampling regulations. He or she should fill in the sampling plan and sampling results, and it is field supervisor' responsibility to keep surveillance and fill in the supervision record. Responsibilities of the sampling staff are: the technical director organizes and determines the sampling plan for the organization concerned; the synthesizer is responsible for the preparation of sampling necessities and the sample acceptance, record-keeping and storage work; the sampling personnel should prepare the sample according to the sampling requirement, and collect the related data and keep records earnestly to ensure the safety and validation of the samples. The leader of the sampling team take charge in the management work during sampling, and he needs to write the work conclusion. Prior to sampling, the technical director should organize the related staff to make a detailed sampling plan according to the testing item and requirement of the entrusted organization, and then verified and approved. The technical director calls sampling personnel together for a meeting to arrange the tasks and explain the sampling requirements, the working contents and the work discipline to them. There should be no less than two experienced staff in each sampling team, and a team leader is appointed to be responsible for the field sampling management. The synthesizer is responsible for the preparation of containers, instrument, sampling list, seal, files, technical standards and letter of introduction needed for sampling. Sampling personnel needs to get the stuff and files mentioned above and takes sample according to the sampling plan.

The sampling staff should keep record of the data and operations relating to sampling which is an integral part of the testing. Sampling record should include the sampling procedure, the identification of sampling personnel, the environment condition, and the site map of the sampling locations as appropriate. There should be no less than two personnel taking part in field sampling. The record kept in field should be clear, detailed, integrated, and the sampling personnel and the representative from the client should sign on the sampling list together. The sampling personnel seal the sample in situ according to related regulation, and signature of the

representative from the entrustment organization may be needed as possibly. Once the samples are sealed, no one is permitted to change or make a replacement. And the sampling personnel should strictly follow the work principles to ensure the authenticity, unbiased and representativeness of the sample.

Instrument manager: Yuan Lin

He is responsible for the maintenance of the instruments. Keep operation record and maintenance record of the instrument. He also takes charge in the calibration of the instrument and preparation and custody of the instrument record card.

Document manager: Liu Liang

The document manager is responsible for the classification, cataloging and custody of the documents. He is responsible for the filing and managing of test reports and documents related. One of his other responsibilities is to file and manage the technical documents such as standards, regulations, procedures and system documents, and the personnel technical documents as well. Moreover, he is asked to keep the file room safe and clean and make sure that the documents in good conditions. He is also the sample keeper and responsible for the classification and record of the sample. He is responsible for keeping the environment of the sample room in normal condition and he should make the sample room safe and clean and also keep the samples in good condition. He is responsible for the distribution of the test reports in time.

Synthesizer: Yuan Lin

He is responsible for preserving the testing samples in right conditions taking the requirements of the client into consideration. He is responsible for storage and management of the consumables. He is also responsible for supervising and inspecting the storage and dispose situations of dangerous goods. And he is responsible for acceptance of the custom’s testing samples and appendices and keeping record of the status characteristics of those; responsible for the test work for the external custom, sample number and status identification; store the samples required to be held in time and keep the availability and integrity of the sample in the storage period. He is responsible for the custom service, getting access to the

requirements of the custom and satisfying their needs. In addition, he takes the responsibility for delivering the feedback information to the person related in order to improve the quality management system. He is in charge of dealing with the complaints and he is asked to summarize the requirements of the custom and report to the quality manager in time. He is responsible for the preparation of the facilities and environmental conditions for test. He is responsible for compiling test reports, and then the test reports are stamped and delivered by him.

The lab supervisor should ensure that the staff is qualified for performing the specialized equipment operation, testing, result assessment, test report sign and certificate verification. If anyone who has not completed training is to be assigned to finish one task, he or she will be supervised according to the Supervising Work Control Procedures. For those who undertake specialized work, there should be qualification confirmation corresponding to their education, training, experience, specific test requirements and certifiable skills. The specific requirements are as follows: for those people who are color blindness should not undertake the tests concerning color identification. For those people who undertake the biotic experiments should know well about the knowledge of bio-test safety operation and sterilization. The chemical parameter authorized signatory should have the undergraduate degree or above in chemical, and moreover, he or she should have the technical working experience for three years at least. If not, he or she should have worked in the chemical related field for at least 10 years.

A5. Project Background

With the rapid development of the world trade and global tourism, the demand for freedom trade is growing and the marine shipping industry is exuberant and occupies 60% share of world trade. To assure the safety of sailing ships, it is necessary to add some ballast to keep the ship in an appropriate stable and floating status. Since 1980s, it is common to use water as ballast, and it is the so called ballast water. While the ballast water makes it easier for the spread of species from one water region to

another one. Once ballast water in ship containing harmful aquatic organisms or pathogens is discharged to the waters of another port state, it will endanger the local ecology, economy and human health, and the effect will last for a long time. Once the aquatic organisms invade and inhibit in the local waters, they will reproduce in an uncontrollable manner, then destroy the food web of local species. And these disastrous causes will lead to mass propagation of harmful parasite and pathogen and even extinguish the local species.

The test and management of ballast water is getting more and more urgent as the ocean pollution is getting worse and worse due to the discharging of the ship ballast water. Aiming to prevent the potentially devastating effects of the spread of harmful aquatic organisms and pathogens carried by ships' ballast water from one region to another. IMO proposed and adopted the International Convention for the Control and Management of the Ship’s Ballast Water and Sediments. Convention stipulates that:(1) the average density of organisms greater than or equal to 50 micrometers in minimum diameter in the replicate samples is less than 10 viable organisms per cubic metre; (2) the average density of organisms less than 50 micrometers and greater than or equal to 10 micrometers in minimum diameter in the replicate samples is less than 10 viable organisms per millilitre; (3) the average density of toxicity *Vibrio cholerae* (serotypes O1 and O139) is less than 1 cfu per 100 milliliters, or less than 1 cfu per 1 gramme (wet weight) zooplankton samples; (4) the average density of *E. coli* in the replicate samples is less than 250 cfu per 100 milliliters; (5) the average density of intestinal *Enterococci* in the replicate samples is less than 100 cfu per 100 milliliters.

A6. Project/Task Description

A6.1 Description of OceanDoctor BWMS

OceanDoctor BWMS is mainly composed of a filtration unit, a photo-catalytic reaction unit, a control unit and the sampling facility.

All units of the BWMS could flexible assembled as required. They can either be

installed on a common skid which is fit for new building ships or installed separately in conformity with the real installation space of the ships which is fit for existing ships. The general arrangement drawing of the system is as shown in figure 3.

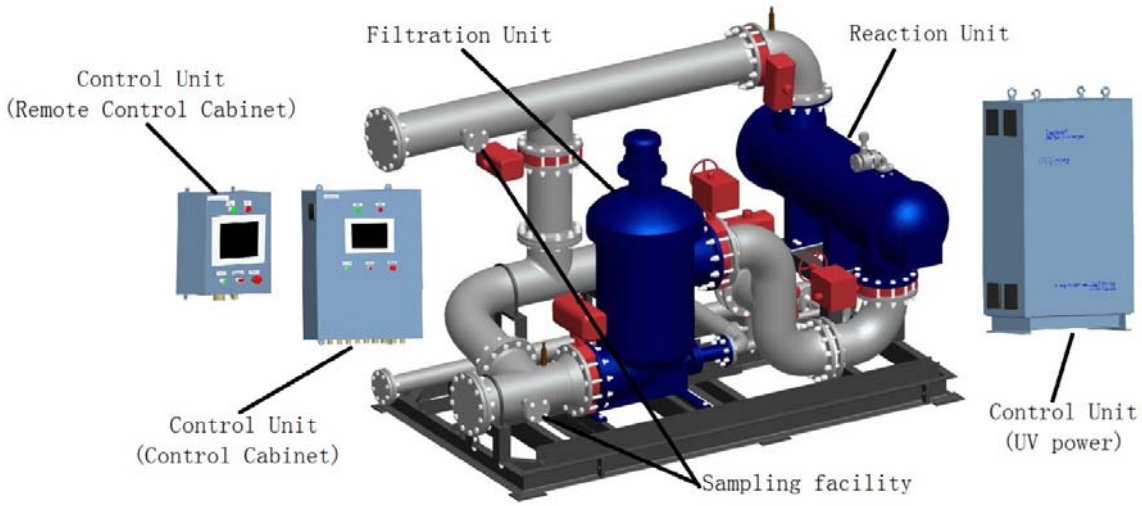

Figure 3 OceanDoctor BWMS

A6.2 the test ship and the test system

A6.2.1 the test location and the test set-up

The shipboard test will be conducted on the vessel named ShunAn 328. This vessel is a bulk cargo ship in operation and mainly navigates along the China coastal route. This ship is fitted with a complete ballast system which including a ballast pump with capacity of 1000m^3/h, ballast pipes, four ballast tanks and pipeline valves, the ship could pump sea water to the ballast tank and discharge ballast water to sea.

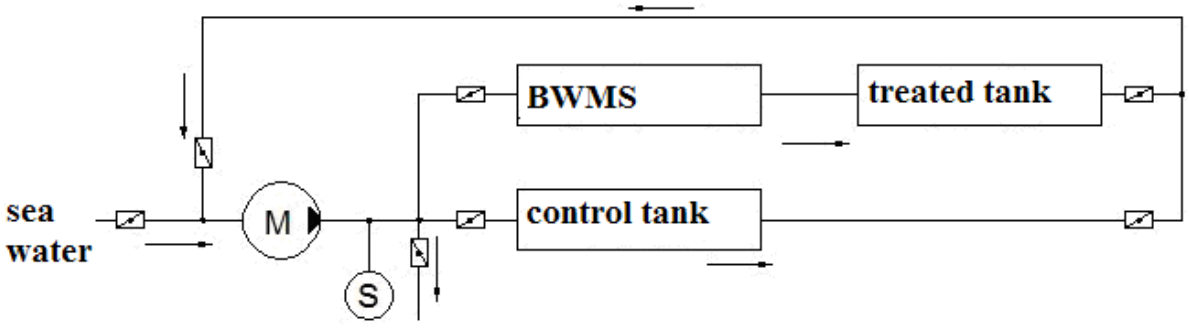

Figure 4 Diagrammatical Drawing of the Test Setup

Connect the model HBS-250 BWMS and model HBS -500 BWMS in parallel to

the existing piping system. The test set-up is able to realize ballasting and deballasting. The ballast water management system will treat the ballast water at uptake, the treated ballast water flows to the ballast tank. And at the same time, some untreated ballast water is pumped to the control tank. When the deballasting is to be conducted, the treated ballast water and the control ballast water will be discharged by the ballast pump outboard. Valves are installed on both the pre part and post part in the pipe of the ballast water treatment system and the existing pipe to enable the pipe shift. A sampling facility is designed according to the requirement of G2 and installed on the pipe to take representative ballast water sample.

The design of the sampling facility meets the requirements as prescribed in G2. the structure of the sampling facility is shown in figure 5.

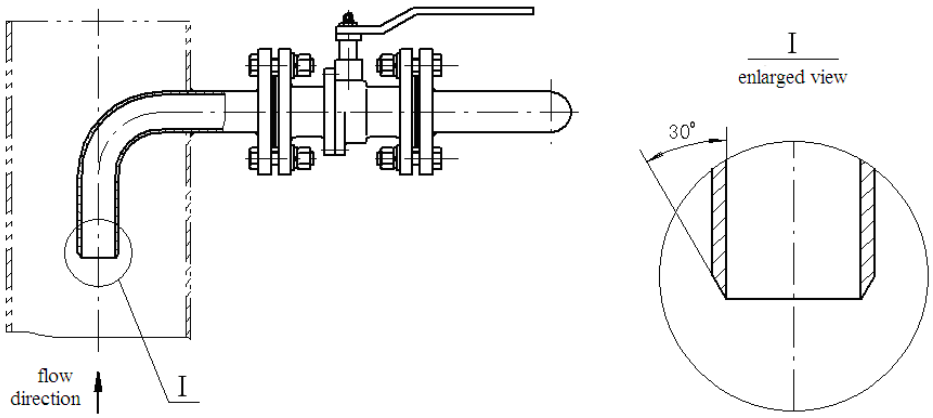

Figure 5 structure of the sampling facility

A6.2.2test system

The specifications of the systems to be tested onboard ship are as follows:

A6.2.2.1 the base unit

Model HBS-250 OceanDoctor BWMS

Treatment Rated capacity（TRC）:250m^3/h

A6.2.2.2 the Scaled unit

Model HBS-500 OceanDoctor BWMS

Treatment Rated capacity（TRC）:500m^3/h.

A6.3 test schedule

The test schedule will be build up upon the assignment of the agreement, and the test procedures will be strictly following the test schedule.

Test schedule（table 1）:

Table 1 Project Test Schedule

date / item	April 2013	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.
QAPP	√								
Test preparation	√								
System commissioning	√								
Test cycle	√	√	√	√	√	√	√		
Sample collection	√	√	√	√	√	√	√		
Data determination	√	√	√	√	√	√	√		
Data analysis							√	√	
Test report							√	√	
conclusion							√	√	

The determination of the testing parameters is in accordance with the test objectives and the schedule of the project. See details in project parameters table 2:

Table 2 List of Test Parameters

No.	Test item	No.	Test item
1	Particulate Organic Carbon（POC）	6	viable organisms ≥50 μm
2	Total Suspended Solids（TSS）	7	*Escherichia coli*
3	salinity（PSU）	8	*Intestinal enterococci*
4	temperature（T）	9	*Vibrio cholerae*
5	viable organisms :10-50 μm	10	Heterophic bacteria

A7. Objectives of Testing Result

The objectives of the test data are to ensure the objectiveness and accuracy of the determined water quality parameters (environmental parameters) and organisms' parameters, and also keep the standard deviation falls within the controlled range. The ballast water onboard ship will be treated by the OceanDoctor BWMS, after treatment, the treated ballast water will be held on ship for some time as determined by the navigation route. Then the discharge of the treated water is to meet the standard as set out in regulation D-2 of the "International Convention for the Control and Management of Ships' Ballast Water and Sediments," that is the ship must discharge: less than 10 viable organisms per cubic meter greater than or equal to 50 μm in minimum dimension; less than 10 viable organisms per milliliter less than 50 μm in minimum dimension and greater than or equal to 10 μm in minimum dimension; Toxicogenic *Vibrio cholerae* (O1 and O139) with less than 1 (cfu) per 100 milliliters; *Escherichia coli* less than 250 cfu per 100 millilitres; *Intestinal enterococci* less than 100 cfu per 100 milliliters. And moreover, for both the control tank and ballast water to be treated, with viable organism concentration exceeding 10 times the maximum

permitted values in regulation D-2.1 and control tank viable organism concentration exceeding the values of regulation D-2.1 on discharge.

The source water for test cycles shall be characterized by measurement of salinity, temperature, particulate organic carbon and total suspended solids.

A8. Test Training Assurance

A8.1 Sample Collection and Handling

The sampling personnel and the sample preparation personnel should be trained in accordance with the Guidelines for Ballast Water Sampling (G2) and the Specification for Marine Monitoring GB17378.4,7-2007. The way to determine the training needs and person to be trained should meet the requirements of the Personnel Training Procedures of Ballast Water Detecting Lab of Shanghai Ocean University. The training plan should be made with reference to the project tasks currently and expected. The availability of the training is to be evaluated. During the training, the trainer should get to know every step of the sample collecting and preparing. The trainer should be supervised by the experienced staff in the lab to conduct the sample collecting and preparing work.

A8.2 Lab Test and Analysis

Staff in the lab involved in the test and analysis task should be trained according to the Personnel Training Procedures of Ballast Water Detecting Lab of Shanghai Ocean University; Specification for Marine Monitoring GB17378.4-2008, GB17378.7-2008; Water quality. Determination of free chlorine and total chlorine: Spectrophotonetric Method Using N,N-diethyl-1,4-phenylenediamine HJ 586-2010; Observation Method for Gulf Ecosystem, Standard Examination Methods for Drinking Water—Indicator Microbes GB/T 5750.12-2006; Diagnostic Criteria for Cholera WS 289-2008, Water quality - Detection and Enumeration of intestinal enterococci - Part 2: Membrane Filtration Method (ISO 7899-2:2000); Guidelines for Approval of Ballast Water Management Systems G8; Procedure for approval of ballast water

management systems that make use of active substances G9. Those workers who will undertake the chemical related work in the lab should learn how to protect and rescue themselves. The important chemical test staff (those who knows well of the test methods, procedures, objectives and result assessment) should master the assessment method for determination of uncertainty of environmental parameters analysis. The personnel who are responsible for organism test should know the safe handling and sterilization procedures of organism test. All the staff should be assured to be qualified and supervised to carry out the work according to the management system.

The lab manager should ensure that the staff is qualified for performing the specialized equipment operation, testing, result assessment, test report sign and certificate verification.

A9. Documents and Records

A9.1 QAPP

The development and research organization and the test organization will discuss and determine the QAPP prior to the implementation of the project. And the QAPP will be handled and recorded as the project controllable document.

A9.2 Field Sampling Record

Information about the collected sampling data will be recorded on the water proof table and the information in table is about the date and time, the sampling location, the sampling personnel, weather, sampling methods, environmental condition, the kind and species of the sampled organism, the sample lot number, the in situ description of the status of the sample and the identified sample quantity. Any deviations from the standard sample procedure or emergencies should all be recorded.

Illustration of one sample label（Figure 6）:

Sample ID:	name of vessel:
Sampling time:	sampling temperature:
Sampling humidity:	No. of the ballast tank:
Sampling Personnel:	supervisor:

Figure 6 Sample Label

The samples collected will be named by standard identification serial numbers;

The ID code of the sample is described as: SHOU-BWDL-JPMT (Acronym of the sample deliverer) –tri-digit serial number +abbreviation of the test classification +the order of the parallel. The abbreviation of the test items are as follows: A represents viable organism sample with a dimension of 10~50μm; B represents viable organism sample equal and greater than 50μm; C represents micro organism sample; D represents environmental parameter sample; for example: SHOU-BWDL-JPMT-001A1 represents the first replicate sample among the first batch of samples with a dimension of 10~50μm viable organisms delivered by Jiujiang Precision Measuring Technology Research Institute and tested by ballast water detecting lab of Shanghai Ocean University.

Table of sampling record（table 3）:

Table 3 Record of Sampling Result

No:_____

Sample name			
Vessel name		client	
Sampling personnel		Sampling date	
Quantity of samples		Environmental factor	Temperature: ℃ Humidity: %RH
Port of registration		Vessel's IMO No	
Vessel tonnage		Ballast water capacity	
Ship's construction date		sample ID code	
Type and location of the sample tank		Type of ballast water management undertaken	
Composition of the BWMS		Sampling approach	
Capacity of the sample tank		other sampling technique adopted	
Sample type（bigger organism/smaller organism, bacteria		net（including depth of the vertical net haul, net opening size, mesh size）	
pump （ sampling depth, pumping capacity in 1/min）		bottle（sampling depth, bottle capacity , unit: l）	
Sampling start time		Sampling end time	
Sample volume		Location of sampling access point（uptake / discharge）	
Type of sampling access point （uptake/discharge）		Size of the sieve for concentration of sample (μ m)	
sampling result	recorder /date:		

A9.3 record of the chain of custody

To assure the quality control of the project, the record of the chain of custody should be kept. Table of chain of custody record, see table 4:

Table 4 Record of Chain of Custody

No:_____

Test No.		Custody date	
Test item		Custody personnel	
Test objective			
Test personnel			
Custody content			
Contract (letter of authorization)	Completely filled □ not complete□ no□		
Test plan	complete□ to□ no□		
instrument	Calibration status qualify□ allow to be used□ forbidden to be used□		
personnel	qualification Hold qualification card□ no qualification card□		
sample	quantity		
	Appearance quality qualified□ not qualified□		
	Unique marking have □ no □		
	Sample disposal record have □ no□		
Test method	Operation document right□ not complete□ to be decided□		
	standard（regulation） have□ no□		
environment	temperature ℃	humidity %RH	
	condition qualify□ not qualify□		
record	Original record meet the requirement□ not meet the requirement□ no□		
	Instrument operation record meet the requirement□ not meet the requirement□ no record□		
Response for custody result			
remark			

A9.4 Laboratory Original Data Records

The original data in the lab must be recorded clearly, and the records will be stored in an appropriate facility to keep them away from damage or losing, and also easy to be accessed. Preservation period of the record must be specified and all the records should be kept secret. The collection, retrieval, access, file, storage, maintenance and cleaning of the quality record and technical record should be in compliance with the Control Procedures of Ballast Water Detecting Lab of Shanghai Ocean University.

The lab should preserve the detailed records of the information about original observation, educe of data, verification route, calibration records, personnel record and the copies of report distributed within the stipulated preservation period. Each test record or calibration record should include the following information: sample identification number, test date, standards, test conditions and so on. This could help to identify the factors of uncertainties and assure the repeatability of the test or calibration in conditions simulating to those of the original. The content of the records should involve information about the name of the sampling personnel, the test personnel, verification personnel. If culture medium is to be prepared, it is necessary to make a record of the name and type of the culture medium; marks of the cultivation time and personnel in charge of the cultivation; type and volume of the culture medium/solution; volume of the sub-package; composition, content, manufacturer and lot number of each composition,; pH value (initial and final); complementing means, time and temperature of sterilizing measures and etc should be recorded.. Keep record of the observation result, data and calculations, and. Identify the records according to the requirements of specified tasks.

If the lab records need to be modified, two lines should be written on the original records, and don’t erase the original records. Then the modified records should be written near the original records with the mender’s stamper or signature or abbreviative signature.

Table of original record of the phytoplankton test（table 5）:

Table 5 Original Record of the Phytoplankton Test

No:_____

Project title			Project No.		
Entrustment date			Test date		
Sample ID number			Sample description		
Applicable standard	The specification for marine monitoring—Part 7:Ecological survey for offshore pollution and biological monitoring GB17378.7-2007/5.3.2.3;instruction sheet of phytoplankton				
Test condition	temperature: ℃,humidity: %RH				
instrument	Phyplanton counting chamber,optical microscope（ ）,cover glass,pipettor				
Sample receiving time			Concentrated constant volume mL(V_1)		
Sampling volume L（V）			Test starting and ending time		~
Test result					
The first glass	Test volume (V_2):	The second glass	Test volume(V_2):	The third glass	Test volume(V_2):
Species name（Latin）		Species name（latin）		Species name（latin）	
Total count（cell）X		Total count（cell）X		Total count（cell）X	
density（cell/ml）D_1		density（cell/ml）D_2		density（cell/ml）D_3	
Average density（cell/ml）D^1					
Note: density（D）=$X*V_1/(1000V*V_2)$ Average density（D^1）=$(D_1+D_2+D_3)/3$					

Table of original record of the zooplankton test（table 6）:

Table 6 Original Record of the Zooplankton Test

No:_____

Project title		Project No.	
Entrustment date		Test date	
Sample ID number		Sample	
Applicable	National standard of People's Republic of China GB17378.7-2007, part 7		
Test contion	temperature: ℃,humidity: %RH		
instrument	optical microscope（ ）, stereo microscope（ ）		
Sample receiving date		Test starting time	
Volume of filtrated water in m^3		Test ending time	
Test result			
species name		quantity	
Total plate count（cell）			
Total density（cell/m^3）			
Note :total density（cell /m^3）=total count（cell）/ volume of filtered water（m^3）			

original record of the bacteria testing（ see table 7a, table 7b, table7c）:

Table 7a Original Record of the test of the Total Plate Count and the *Escherichia coli*

sample name:

date of received: date of test:

Test location and ambient condition: microorganism detection room temperature: ℃ relative humidity: ％RH

Test instrument: cylinder (100ml), clean bench, two Pipettes (0.1ml and 1ml),timer, incubator, spreading rod

Test method: GB /T 5750.12. 1,2.1,2.2,4.1,4.2-2006;GB 17378.7-2007.10.1,9.1;Instruction Sheet of Microorganism Test

Testing results and records:

（1） Total plate count（fresh water）: pour nutrient agar into each plate of the decimal scale potency dilution samples, and add blank.

（2） Total plate count（seawater）;take 0.1ml of bacteria sample at each dilution gradient to spread it to the 2216E plate, add blank, then incubated the plate at 36℃ ,then examined after 7 days. If sample concentration is relatively low, pipette 1 mL of water sample and place it to the plate.

（3）total coliforms（Multiple tube fermentation method）;five double lactose peptones (each with a volume of 10ml) are incubated at 37℃ for 24h. If any acid or bubble is generated, vaccinate the Eosin methylene blue plate, and make it incubated at 37℃ for 24h, choose the suspicious colony for Gram staining and inoculate the Lactose peptone at the same time, and make it incubated at 37℃ for 24h. Referment to generate acid and bubble. Gram-negative no bacillus gives a positive result in coliform group test. If it is gram-negative budless bacillus, then we say the result is bacteria positive.

（4）Escherichia coli（Multiple tube fermentation method）; vaccinate the positive tube as mentioned in(2) to EC-MUG at 44.5℃ for 24h, observe under the UV lamp, The presence of bright blue fluorescence is considered a positive response for E. coli.

（5） total coliform（membrane filtration method）;100ml of water sample is filtered(in the condition that the concentration of the water sample is relatively high, dilute the water sample by two gradient） on the germ free membrane, place the membrane on the Fuchsin sodium Sulfite culture medium at 37℃ for 24h. Choose the suspicious colony for gram staining, and inoculate the Lactose peptone at the same time at 37℃

for 24h. referment for observation of acid or bubble, Gram-negative no bacillus gives a positive result in coliform group test.

（6）*Escherichia coli*（membrane filtration method）;inoculate the positive membrane (mentioned in (4)) to theNA-MUG, incubated at 37℃ for 4h, observe under the UV lamp, The presence of bright blue fluorescence is considered a positive result.

Sample ID code	total plate count（cell/mL）						Total coliform（MPN/100mL）（CFU/100mL）		*Escherichia coli*（MPN/100mL）（CFU/100mL）	
	undiluted	10^{-1}	10^{-2}	10^{-3}	10^{-4}	result		result		result
SHOU-BWDL-										
SHOU-BWDL-										
SHOU-BWDL-										
SHOU-BWDL-										
SHOU-BWDL-										
SHOU-BWDL-										
SHOU-BWDL-										
SHOU-BWDL-										
SHOU-BWDL-										

Blank control: positive control:

Total plate count: Total coliform:

test personnel: reviewed by:

date: date:

Table 7b Original Record of Vibrio cholerae Testing

Sample name;

Date received; date of testing;

Test location and ambient condition: microorganism detection room temperature; ℃ relative humidity; ％RH

Test instrument;cylinder, clean bench, Pipette, timer, incubator at 37℃.

Test methods;WS 289-2008 appendix A

Testing results and records;

A volume of 450ml Water sample is collected,

add 0.5ml of phenothalin(1％) and 0.3ml of potassium tellurate. Adjust the pH to 8.4 -9.2 with 1 mol/L of NaOH..

Add 50mL of ten times of APW, after cultured in the incubator for 6h at 37℃, inoculate to the TCBS plate at 37℃, and then examined after 24h, visually observe if there is growth of suspicious colony.

Sample ID;SHOU-BWDL- yes（ ）,no（ ）whether there is growth of suspicious colony

sample ID;SHOU-BWDL- yes（ ）,no（ ）whether there is growth of suspicious colony

sample ID;SHOU-BWDL- yes（ ）,no（ ）whether there is growth of suspicious colony

sample ID;SHOU-BWDL- yes（ ）,no（ ）whether there is growth of suspicious colony

sample ID;SHOU-BWDL- yes（ ）,no（ ）whether there is growth of suspicious colony

sample ID;SHOU-BWDL- yes（ ）,no（ ）whether there is growth of suspicious colony

sample ID;SHOU-BWDL- yes（ ）,no（ ）whether there is growth of suspicious colony

sample ID;SHOU-BWDL- yes（ ）,no（ ）whether there is growth of suspicious colony

sample ID;SHOU-BWDL- yes（ ）,no（ ）whether there is growth of suspicious colony

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test personnel; reviewed by;

date; date;

Table 7c Original Record of Intestinal enterococci Testing

Sample name;

Date received; date of testing;

Test location and ambient condition: microorganism detection room temperature; ℃ relative humidity; ％RH

Test instrument; cylinder, clean bench, Pipette, timer, incubator (37℃, 44℃)

Test methods: ISO 7899-2: 2000

Testing results and records:

100ml of water sample is filtered by a germ free filter(dilution operation might be conducted when the concentration of water sample is relatively high), place the filter membrane on the Intestinal enterococci culture medium by membrane filtration method, the plate is culture at 37 for 44h. Observe if there is any growth of colonies in red, nut brown or pink.

If there is any typical colony, transfer the filter membrane to the bile esculin azide agar plate which has been preheated to 44℃.under this condition the colony is cultured for 2h at 44℃. Then observe the plate, if the color of the culture medium around the colonies is brownish black, it means the colonies are positive, these colonies is counted as *Intestinal enterococci*.

sample ID;SHOU-BWDL- yes（ ）,no（ ）whether there is growth of suspicious colony; total count of positive colony

sample ID;SHOU-BWDL- yes（ ）,no（ ）whether there is growth of suspicious colony; total count of positive colony_____

sample ID;SHOU-BWDL- yes（ ）,no（ ）whether there is growth of suspicious colony; total count of positive colony_____

sample ID;SHOU-BWDL- yes（ ）,no（ ）whether there is growth of suspicious colony; total count of positive colony_____

sample ID;SHOU-BWDL- yes（ ）,no（ ）whether there is growth of suspicious colony; total count of positive colony_____

sample ID;SHOU-BWDL- yes（ ）,no（ ）whether there is growth of suspicious colony; total count of positive colony_____

sample ID;SHOU-BWDL- yes（ ）,no（ ）whether there is growth of suspicious colony; total count of positive colony_____

sample ID;SHOU-BWDL- yes（ ）,no（ ）whether there is growth of suspicious colony; total count of positive colony_____

sample ID;SHOU-BWDL- yes（ ）,no（ ）whether there is growth of suspicious colony; total count of positive colony_

test personnel; reviewed by;

date; date;

original record of environmental parameters testing

（table 8）:

Table 8 Original Record of Environmental Parameters Testing

Project No: No:_____

Project title							Test parameter						
Entrustment date							Sample date						
Sample description													
Applicable standard							.						
Test condition				temperature: ℃			Test method						
Test preparation													
instrument													
Instrument model													
Test date													
Test time													
Test result													
No.	Water depth	Sampling point	Sample ID code	determined value				Water temperature in situ t_w/℃	calibration				In situ pH_w
				1	2	3	average						
remark													
Calculation method													
No. of test report													
Tested by									Reviewe				

B. Project test and Data Acquisition

B1. Test Preparation

B1.1 Test Condition Preparation

The technical director will take charge in dividing the staff into the sampling group and the test group, and assign task for each team. She is responsible for preparing the related regulations, standards and operating instructions according to the testing task. To determine the instruments, apparatus and the environmental conditions, and purchase and check the chemical reagents and consumables needed for experiment is also one of her responsibilities. Preparation of such stuff as the blank records, sampling bottles, sample labels, sampling facilities, and sample handling reagents and so on is one of her responsibilities as well.

Check all the technical specifications of the treatment system and get ready for the performance test to the treatment system.

B1.2 Test Items and Test Methods

The lab will conduct the following tests: test of four environmental parameters, heterotrophic bacterium, *Escherichia coli*, *Intestinal enterococci*, Toxicogenic *Vibrio cholerae* （serotypes O1and O139）and organisms with two kinds of dimensions according to the *Guidelines for Approval of Ballast Water Management Systems (G8)*, and the requirements of the project entrustment organization. Refer to table 9 for the detailed testing items and methods.

Refer to table 14 for the calibration requirements of the major instruments that will be used in the test.

Table 9 Testing Items and Methods

Test items	No.	Test particulars	Test method
Microbes	01	*Escherichia coli*	The specification for marine monitoring Part 7:Ecological survey for offshore pollution and biological monitoring GB17378.7-2007/9.1,9.2
	02	*Vibrio cholerae*	Diagnostic criteria for cholera WS 289-2008
	03	*Intestinal enterococci*	Water quality Detection and enumeration of *Intestinal enterococci* Part 2: Membrane filtration method BS EN ISO 7899-2:2000
	04	Heterotrophic bacteria	The specification for marine monitoring Part 7:Ecological survey for offshore pollution and biological monitoring GB17378.7-2007/10.1
Phytoplankton	05	Phytoplankton	The specification for marine monitoring—Part 7:Ecological survey for offshore pollution and biological monitoring GB17378.7-2007/5.3.2.3
zooplankton	06	zooplankton	The specification for marine monitoring—Part 7:Ecological survey for offshore pollution and biological monitoring GB17378.7-2007/5.3.3.3
Environmental parameters	07	TSS	GB17378.4-2007/27 The specification for marine monitoring—Part 4:Seawater analysis GB17378.4-2007/27
	08	Temperature	The specification for marine monitoring—Part 4:Seawater analysis GB17378.4-2007/25.1
	09	POC(mg/L)	Gulf ecosystem observation method China Environmental Science Press 2005 / 4.5.14.1
	10	salinity	The specification for marine monitoring—Part 4:Seawater analysisGB17378.4-2007/29.1

Table 10 List of Test Instruments Allocation

No.	Test particulars	Main instrument used	models	Measurement range	j expand uncertainty /k largest tolerance /l level accuracy
01	Heterotrophic bacteria	GZX-Ⅲserial light incubator	GZX-400BS-Ⅲ	（0～60）℃	*U*=0.3℃, （*k*=2） ±0.2℃
02	*Escherichia coli*	GZX-Ⅲserial light incubator	GZX-400BS-Ⅲ	（0～60）℃	*U*=0.3℃, （*k*=2） ±0.2℃
03	*Vibrio cholerae*	GZX-Ⅲserial light incubator	GZX-400BS-Ⅲ	（0～60）℃	*U*=0.3℃, （*k*=2） ±0.2℃
04	*Intestinal enterococci*	Model LDZX vertical pressure steam sterilizing pot	LDZX-75KBS	（50～126）℃	*U*=0.5℃, （*k*=2） ±0.2℃
		Model DK electric-heated constant temperature water bath kettle	DK-S26	（RT+5～99）℃	*U*=0.3℃, （*k*=2） ±0.3℃
05	Phytoplankton	biological microscope	S8APO	（40～1600）X	±5%
06	Zooplankton	biological microscope	DM500	（40～1600）X	±5%
		stereomicroscope	PXS	（10～80）X	±5%
07	TSS	electronic balance	AL104/01	（0.0001～110）g	I 级
08	Temperature	thermometer	（0～40）℃	（0～40）℃/0.2℃	0.62℃
09	DOC	UV/VIS Spectrophotometer	UV-2000 型	（190～2600）nm	IV 级
10	salinity	salimeter	SYA2-2	2～42	*U*=0.0038 （*k*=2）

B2. Test and Sampling planning

B2.1performance test

The performance test will be conducted in accordance with G8. The test will last for 6 months and three consecutive, valid test cycles will be finished. Firstly, the ballast tank will be subdivided into the control tank and the treatment tank. The procedures of the test cycles are given as follows:

1） the uptake of the ballast water of the ship to the treatment tank

When the ship is about to startup the ballast operation, the inlet valve of the treated tank and the valves both at the front and the back of the BWMS are opened, and the valve on the bypass pipe is closed. And then the ballasting status of the BWMS is started, and the ballast pump will be started at the time when the feedback signal “ready” is received, The seawater will be treated by the system and the treated water will flow into the ballast tank. In this process mentioned above, the BWMS is of a capacity within the range of the treatment rated capacity for which it is intended.

2） the uptake of the ballast water of the ship to the control tank

open the inlet valve on the control tank and the valve on the bypass pipe, close the valves on the front and the back of the BWMS. Then, the ballast pump is started and the seawater is pumped to the control tank and stored. The ballast pumped will be shutdown as required or shifted to other ballast tanks.

3） storage of the ballast water on the ship

when the ballasting is over, the ballast water will be stored in the treated tank and the control tank for a time phase determined by the ship’s navigation arrangement.

4） discharge of ballast water from treated tank from the ship

When the ship is about to start the deballast operation, the outlet valve on the treated tank and the valve on the bypass pipe are opened. And then close the front and the back valves on the BWMS, start the deballasting status of the BWMS. The ballast pump is started when the feedback signal “ready” is received. The water in the treated tank will be discharged to the sea after that.

5） discharge of ballast water from the control tank from the ship

Open the outlet valve on the control tank and the bypass valve on the bypass pipe, and close the front and the back valves on the BWMS. then the water in the control tank will be discharged to the sea.

B2.2 Sampling Facilities and Sampling points

In line sampling method is adopted, isokinetic sampling is realized with the help of the“isokinetic” sampling facility installed on the uptake and the discharge line. The ballast water is pump from the sea to the pipe. For the treated tank, water sample S-1 is collected from the sampling point at the uptake. For the control tank, water sample S-2 is collected from the sampling point at the uptake. The test cycle is finished when the treatment tank and the control tank are filled and then the both tanks are closed. After the ship finished its navigation, the ballast water in the treated tank will be discharged outboard. Discharge water sample S-3 is taken from the discharge line. For the control tank, collect the discharge control water sampling S-4 at the sampling point located in the discharge line.

The sampling facilities are designed for compliance with G2. For the isokinetic sampling, a quantitative water sampler is applied to collect the sample. double layer plankton net with a mesh size of 50μm is used to collect samples of organisms greater than 50μm in minimum dimension. For organism (10μm~50μm) samples and the environmental parameters sample , the samples are collected by a quantitative water sampler.

For collecting the bacteria sample, using the sterilized sampling bottle by way of fire ring sealing sampling. The arrangement of the sampling points is as shown in figure 7.

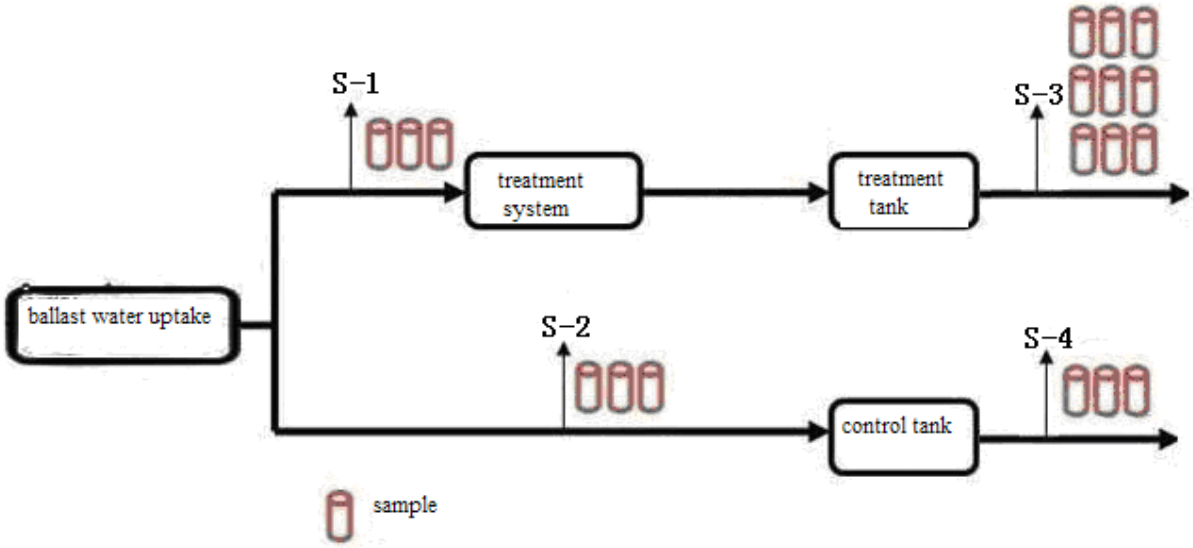


Figure 7 Arrangement of the Sampling Points

The environmental parameters and the organisms and bacteria will be determined based on the water samples collected at the sampling points. Water samples of organism ≥50μm, organisms 10μm~50μm, environmental parameters and the bacteria will be taken at the sampling S-1, S-2 at different time period during ballast uptake (beginning, middle, end). The discharge samples are collected at the sampling point S-4. samples for testing of each type of testing will be collected separately, the sampling location should be at a location on the straight part of the discharge line as near to the control tank as practicable, and where the flow in the discharge pipe is fully mixed and fully developed, and the flowing stream at the sample point is representative of the contents of the discharge ballast water. Three replicate samples will be collected at beginning, middle and end during deballating, totally nine samples. The sampling point of those samples should be at a location on the straight part of the discharge line as near to the ballast water discharge overboard as practicable, and the flowing stream at the sample point is representative of the contents of the discharge ballast water. The list of the sampling size and the quantity of samples are shown in table 11.

Table 11 Sampling Size and Quantity in Each Sampling Point

Test particular	S1		S2		S3		S4	
	Sampling size	quantity	Sampling size	quantity	Sampling size	quantity	Sampling size	quantity
Organisms ≥50μm	1m^3	3	1m^3	3	1m^3	3×3	1m^3	3
organisms 10μm~50μm	10L	3	10L	3	10L	3×3	10L	3
bacteria	1000ml	3	1000ml	3	1000ml	3×3	1000ml	3
Environment parameters	5L	3	5L	3	5L	3×3	5L	3

B2.3 sampling time interval arrangement

Samples are collected over the period of uptake (beginning, middle, end), and samples are collected over the period of discharge (beginning, middle, end). The sampling time period should exclude the time took for washing pipe; the time for washing the pipe is ten minutes before test cycles and ten minutes before discharge.

B3. Sampling Methods

B3.1 sampling facility

A quantitative water sampler, flow meter, plankton net and quantitative sampling bottles are to be used for sampling. Parameters are determined in field using salimeter, thermometer and the environmental factors are measured by a hygrothermograph.

B3.2 Sample Collection

B3.2.1 collection of environmental parameters samples

Total Suspended Solids (TSS), Dissolved Organic Carbon (DOC) water samples are collected at each time interval. Collect 5L in each replicate, the quantity of water samples are listed in table 10.

Particulate Organic Carbon (POC) and total suspended solids (TSS) water samples will be pretreated, placed in the refrigerator and delivered to the Lab for test. The remaining parameters (salinity and temperature) are to be measured in field.

B3.2.2 **collection of organism samples**（10≤size＜50 μm and size ≥50 μm）

B3.2.2.1 organisms（10≤size＜50 μm）

Certain volumes of water samples are collected at each time intervals and Add 2ml/L algae vital staining solution for 15min and add formalin to fix the samples. add some formalin to fix the sample

B3.2.2.2organisms（≥50 μm）

Install an electric flow meter on the outlet, Water samples taken from the outlet at different time intervals will be filtered by a plankton net with a mesh size of 50μm. the concentrated water sample will be collected into a specimen bottle with content of 60ml. After that add five drops of zooplankton vital staining solution to the specimen bottle, staining for 30 minutes and then add the formalin for fixing.

B3.2.3 collection of microorganism samples

A clean and sterilized glass container will be used when taking the microorganism sample. Underwater sampling method is used in order to avoid being polluted. If there is residual chlorine in the water sample, add sodium thiosulfate which is germ free to the glass container(sodium thiosulfate with a volume of 0.1ml and a concentration of 10% added to the water sample of 120 mL will produce a reduction residual chlorine 15mg/L). The volume of the water sample should be no less than 500ml.

B3.3 Sampling/Test System Failure Response and Remedy

Any interruptions or unexpected things occur during sampling should be given a due consideration and causes of them should be found. Report to the technical director in time and keep detailed record of the failure event. If it is resulting from instrument system failure, continue to test by applying another same instrument if

necessary. Or else, repair immediately after report to the technical director.

Once the test is interrupted, cut the power supply in accordance with operation procedures.

There are circumstances when external accidents such as power failure, water failure and so on, happen which will affect the testing quality. Therefore, re-sampling and retest are needed when it returns to normal.

When it happens that the failure of instruments or apparatus causes the test to breakup. If there is a backup instrument, use it to replace the faulty one and continue the test. If there is only one instrument in the lab, and the failure of the instrument will affect the test quality, re-sampling until the instrument returns to normal. The instrument will be handled according to the Procedures for Instrument and Apparatus Management.

If something is wrong with the sample, stop the test and report to the technical director. Check the sample, find out the causes and make a suggestion. After being approved, decide how to solve the problem. Re-sampling and re-test are necessary.

Keep record of all abnormities and interruptions occur in the handling process and fill in the sampling/test process and the result abnormalities handling record table. And report to the technical director.

B4. Sample Handling and Storage

B4.1 Sample Handling in Field

After samples of organisms with particle size between 10μm to 50 μm are collected, add algae vital staining solution with concentration of 2ml/L and add formalin to fix the sample. After the samples are taken back to the lab, make the samples static for 24h and later use a device to absorb and filter the supernatant fluid of the phytoplankton. After 80% of supernatant fluid is filtered, make the sample stay static once again for another 24h. After the sample is secondary absorbed to about 100ml, make it stay static for 24h for the third time, and then a constant volume 50ml

is left for microscopic counting.

After sample of organisms with size greater than 50 μm, add five drops of zooplankton vital stains for 30min, and then add formalin to fix the sample. Then the samples are taken back to the lab, settling down for 24h. After that filter part of the supernatant fluid and remove the samples to the 50ml cone centrifuge tube and keep making it settle down. Finally, filter the supernatant fluid and microscopic count the total number of the organisms.

If there is residual chlorine in the water sample, the samples are pretreated by adding certain sodium thiosulfate which is bacteria free in the glass container (residual chlorine of 15mg/L could be reduced by adding 0.1ml , 10% sodium thiosulfate to water sample of 120ml).

B4.2 Sample Storage, Transportation and Preservation Time

The samples for environmental parameters should be stored in dark. And the TSS, POC should be pretreated onboard and taken back to the lab for test. The organism samples are to be fixed in field and placed in dark and then transported to the lab. The test to them is to be finished within 7days. The bacteria (microorganism) samples are inoculated in field and the culture medium is transported to the lab for test.

After tested, the organism samples can be store for a long term being placed in fixing liquid and dark place. Add some formalin fixing liquid once three months to keep the samples from decay. The bacteria samples should be tested right after transported to the lab for there is no way for long term storage. The environmental parameters samples are not suitable for long term storage after tested, if retesting is necessary, the holding time of the environmental parameters samples should be kept within the valid testing period.

B5. Test Analysis Methods

B5.1 Analysis of Organisms in Water Sample (10μm– 50 μm and ≥ 50μm)

B5.1.1 10-50 μm viable organisms

Lightly absorb the supernatant fluid from the pretreated samples using a suction pipe with 10μm bolting silk after being settled for 24h. After settling down for a few times, the water sample is condensed to a 50ml thimble tube. Shake enough before sampling counting, absorb a certain amount of sample and then release it at the counting chamber covered with cover glass (make sure there are no bubbles remain) and then conduct the microscopic counting(GB17378.7-2007). For how many phyplankton to be counted, all, half or a quarter, it is decided by the number of the phyplankton. Repeat for 3 times for counting of each sample. Do not count the phyplankton cells whose pigment is lost or the remaining counts for less than one half of the cell. Cell which has not finished the cell division is considered to be one cell. Large group of cells or cells in bundle whose quantity is hard to count can be expressed by the grade symbol to indicate the number of it.

Optical microscopic counting (concentrated counting):

$$C = \frac{n \times V_1}{V_2 \times V_n}$$

where:

C——total amount of samples in per unit volume, unit（cells/m^3）;

n——number of samples, unit（cells）;

V_1——the volume of concentrated water sample, unit: ml;;

V_2——volume of filtered water, unit:(m^3);

V_n——volume of sampling counting, unit:(ml).

B5.1.2 viable organims ≥50 μm

The samples being filtered and concentrated are identified and analyzed by total count method and counted by kind/species to calculate the organism number (number of organisms in per unit)(GB17378.7-2007):

$$\gamma_B = \frac{V_B}{V}$$

where:

γ_B——number of zooplanktons in per unit of volume;

V_B——volume of sample, unit(ml);

V——volume of filtered water, unit:(m^3).

B5.2 Environmental Parameter Analysis

B5.2.1 salinity

Determine the salinity in field using the USA kimcheon YSI 85-25 salinometer(GB17378.4-2007).

B5.2.2 water temperature（T）

Measured by reversing thermometer in filed (GB 17378.4-2007).

B5.2.3 Total Suspended Solids（TSS）

Determination of TSS -Gravimetric method: A certain volume of water sample passes 0.45μm membrane, dry and weigh the TSS left on the membrane, and calculate the concentration of the suspended solid in water (GB17378.4-2007).

$$\rho = \frac{W_1 - W_2 - \Delta W}{V}$$

$$\Delta W = \frac{1}{n} \times \sum^{n} (W_n - W_b)$$

where:

ρ——concentration of suspended solids, unit (mg/L);

W_1——weight of suspended solid plus membrane (W_2),unit (mg);

W_2——weight of water sample membrane, unit (mg);

V——volume of water sample, unit (L);

ΔW ——calibration value of blank calibration membrane, unit (mg);

W_n——weight of the blank calibration membrane after filtered, unit (mg);

W_b——weight of the blank calibration membrane before filtered, unit (mg);

n——number of blank calibration membrane;

B5.2.4 Particulate Organic Carbon（POC）

Determination of particulate organic carbon (POC) by spectrophotometry. The carbon is wet oxidized by acidic dichromate; the decrease of the extinction value of the yellow dichromate solution may indicate the quantum of oxidized carbon "observation method for gulf ecosystem". Calibrate the measured extinction value:

$$E = 1.1 \times E_f$$

where:

E_f—difference of extinction value of sample and blank solution

Calculate the concentration of POC, unit: μg/L.

$$\mathrm{POC} = \frac{E \times F \times v}{V}$$

where:

V—volume of filtered seawater sample, L;

v—the volume of oxidation used in the C step;

factor F is calculated as follows:

$$F = \frac{120}{E_3}$$

where:

E_3—calibrated average extinction value of trivalent chromium at 440nm.value of F calculated is about 275.

B5.3 Bacteria Sample Analysis

B5.3.1 **Determination of heterotrophic bacteria in water sample**

Add 1mL of tween-80 solution to per 100mL of bacteria sample solution. Gradient dilution is made by high pressure sterilized seawater. Before the water sample is diluted, shake it with effort to make it mixed sufficiently. 10ml water sample is sucked by a sterilized suction tube and added to 90ml sterile dilution, getting a 10 times of dilution water sample. Shake it to make it well mixed. After that, based on the 10 times degree dilution, make the 100times, 1000times degree dilution in the same way as mentioned above and shake them to be well mixed. When the above mention diluting process is conducted, there is no need to change the sterilized suction tube. Take 0.1ml diluted water sample and spread it uniformly on the 2216E culture medium. Four dilution degrees of each water sample are needed to be prepared and replicate two plates for each dilution degree. Put the plate into a constant temperature culture box (25℃) with its upside down for 7 days. Count the total number of the colonies with a stereomicroscope:

（1） Do not count when large lawn appears on the plate.

（2） Plate with number of colonies between 30 and 300 is selected; the average number of colonies multiplies the dilution degree (10 times,100 times or 1000 times) equals to the number of the bacteria in water sample.

（3） If there are two kinds of dilution degrees with average number of colonies are between 30 and 300, the ratio of the two numbers determines which one to choose. If the ratio is less than 2, the average of the two is chosen; if more than 2, the colony with less number is chosen.

（4） If all the average values of all kinds of degrees of dilution are more than 300, the number of colonies is counted using the average number of colony in the largest degree of dilution (lowest concentration) multiplies the times of dilution.

（5） If all the average values of all different degrees of dilutions are all less than 30, the number of colonies is counted using the smallest degree of dilution (highest concentration) multiplies the times of dilution.

（6） If there are no colonies in all different degrees of dilution, and no inhibitor is tested, then report less than 1 multiplies the lowest diluted times.

B5.3.2 Determination of *Escherichia coli* in water sample

The samples should be tested by multi-tube fermentation method immediately after they are transported to the lab in cool conditions in accordance with The specification for marine monitoring Part 7: Ecological survey for offshore pollution and biological monitoring GB17378.7-2007/9.1,9.2. Shake the water sample over 25 times at least before testing or diluting to make the water sample well mixed. 10ml water sample is sucked by a sterilized suction tube and added to 90ml sterile dilution, getting a 10 times of dilution water sample. Shake it to make it well mixed. After that, based on the 10 times degree dilution, make the 100times, 1000times degree dilution in the same way as mentioned above and shake them to be well mixed. When the above mention diluting process is conducted, there is no need to change the sterilized suction tube. A 10ml of the original water sample is inoculated to the 10ml two fold lactose peptone culture medium; a 1ml water sample is inoculated to the 10ml unblended lactose peptone culture medium. Further more, take 1ml10-1, 10-2, 10-3 diluted water sample to the unblended lactose peptone culture medium, five tubes of each degree of diluted water sample are inoculated. Put the inoculation tube into a culture box with a temperature of 36±1℃ for 24±2 hours. If no bubble or no acid is produced in the lactose peptone culture pipe, it indicates the *Escherichia coli* to be negative. If there is gas or acid produced, follow the steps bellows: inoculate the water sample in the fermentation tube to the eosin methylene blue agar plate, and put it in the 36±1℃ culture box for 18h-24h. Observe the appearance of the colonies, and choose the kind of colony which gets the specific features(dark purple black, metallic luster; purple black, no or little metallic luster; light purple red, dark in the center) for Gram staining, microscopic test and verification test. If the water sample is tested to be Gram negative sporeless bacterium,inoculate to the lactose peptone culture liquid at the same time and put it into the culture box with a temperature of 36±1℃ for 24±2h, if there are gas and acid produced, it is a proof of the existence of the *Escherichia coli*.

Test of the *Escherichia coli* in the tube which has fermentation phenomenon and

gas or acid in it. A metal inoculation loop being sterilized by burning or the sterile cotton swab is used to inoculate the liquid in the tube mention above to the EC-MUG tube. Put the tube which has been inoculated into the culture box with a temperature of 44.5±0.5℃ for 24±2h. The EC-MUG tube is radiated in the dark by a 6 w power UV lamp with a wavelength of 366nm, if blue fluorescent light is observed; it shows that there is *Escherichia coli* in the water sample. Count the number of the positive EC-MUG tubes, refer to the most probable number (MPN) table for the matched most probable number of the *Escherichia coli*, reported the result of the number of *Escherichia coli* in the unit of MPN/100ml.

B5.3.3 Determination of Intestinal Enterococci in water sample

The samples should be tested immediately after they are transported to the lab in cool conditions in accordance with the standards stipulated in water quality- detection and enumeration of the intestinal *Enterococci* ISO7899-2-2000. Shake the water sample over 25 times at least before testing to make the water sample well mixed. connect the sterilized filter device to the Buchner flask, put the membrane at the bottom of the filter with a germfree tweezer, and certain amount of water sample is sucked to the filter, and be sucked and filtered by the vacuum pump. After all the water sample liquid passes through the membrane, clean the edges of the filter with 20ml to 30ml normal saline for twice at least. Then, turn off the vacuum pump and turn on the filter, and take the filtered membrane by a germfree tweezer onto the surface of the mEI agar culture medium- Slanetz and Bartley medium (membrane *Intestinal enterococci* culture medium). Ensure that no bubbles in the middle of the membrane and the medium. Put the plate upside down in temperature of 36°C±2°C for 44±4h. After the culture time is over, all the colonies which is red, nut brown or pink, no matter in the middle or full over the plate are all typical. If there are typical colonies formed, transfer the membrane and the colonies using the germfree sweezer to the Bile Esculin Azide Agar plate which has been preheated to 44°C, culturing for 2h at 44℃±0.5℃, then observe the plate, if the color of the culture medium around the colonies is brownish black, it means the colonies are positive, these colonies is counted as *Intestinal enterococci*.(note: counting when the colonies are uneven

distributed or bulge will affect the identification of the positive colonies. The color will diffuse to the colonies nearby). Count the membranes which are proved to be *Intestinal enterococci* colony, unit:(CFU)/100ml.

B5.3.4 Determination of *Vibrio cholerae* in water sample

The samples should be tested immediately after they are transported to the lab in cool conditions in accordance with the standards stipulated in the Diagnosis Standard for *Vibrio cholerae* WS289-2008. The samples collected are inoculated in the culture medium as soon as possible. Water sample of 450ml is inoculated in each 50ml, 10times concentrated alkaline peptone culture medium and it is put in a temperature of 37℃ for 6-8h. And afterwards, take an inoculation loop culture from the bacteria film undersurface and streaking inoculated to the two kind of culture mediums ,the strong (Gentamycin agar, TCBS agar and No.4 Agar), and weak (alkaline nutrient agar) in37℃ for 18-24h. Cultivate the strain identification of the typical colonies grown on the strong and the weak culture medium (Slide agglutination test, oxidase test and strain review).

The characteristics of *Vibrio cholerae* bacteria grown on different medium are various, the characteristics of colonies grow on the common culture mediums in 37℃ for 18-24h are as follows:

(1)Alkaline nutrient agar: colorless, round, transparent or translucent, surface smooth, wet, flat or bulge slightly, edge neat, diameter of the colony about 2mm.

(2)Gentamycin agar and No.4 Agar: the characteristics of this kind of colonies are similar to those grow on the alkaline nutrient agar. But not so transparent, almost be translucent. And for that there is tellurite in the culture medium, the color of the center of the colony is usually grey or grey black and getting darker and darker as time goes.

(3)TCBS agar: yellow, glitter, surface smooth, wet, bulge slightly, edge neat.

If any questionable colonies found in the Serum agglutination reaction, send the colonies to the Shanghai Luwan District Disease Control Centre for subcontract testing.

B6. Quality Management Plan（QMP）

As the project test organization, the Ballast Water Detecting Lab of Shanghai Ocean University will carry out the project quality management in compliance with the Quality Management Plan QMP and takes part in the comparison of the testing results with those obtained by competent labs specialized in the same testing field, and participates in the proficiency testing program organized by authorized organization according to the lab file which is called the Procedures for Testing Result Quality Control. Retest of the samples in the retention time, retest the same sample by the same method or different method, and retest the same sample using the same instrument or different instrument to assure the quality of the test result. Keep the sensitivity, accuracy, deviation allowance range, precision of the parameters; ensure the reliability and integrity of the data.

Enhance the quality awareness of the test personnel; make a clear division of quality responsibilities. The test undertaking organization should be supervised by the entrustment organization and the technical supervision organization. The test undertaking organization should take the quality control procedures in the test process into the quality operation system and make the quality plan to comply the quality system and requirements of the testing project.

B6.1 Quality Management of Field Sampling

Assure the quality management of field sampling and analysis. Prepare the procedures for conducting the sampling and avoid the samples being polluted. Keep away from the interferences caused by the ship itself or the sampling facility. Select sampler, sample bottles made of material appropriate for the testing items. Take antipollution measures for accessories such as winch, cable, and guide wheel in testing location; minimize the influence of the interface enrichment. The pretreatment of samples should be completed in situ right after the samples are collected, and then add some stabilizer and store in low temperature. Items which are susceptible for

microbes' activities or change fast with time should be finished testing within the stipulated time.

Parameters like salinity, water temperature, should be measured in field. During the field test, turn on the instrument and leave it to be warmed-up until the instrument reading and the flow of the ballast water management system become stabilized, and then wash the sample bottle for two times with a little of water sample, afterwards, fill the bottle up with sample, the probe of instrument in the sample bottle, get the reading after the instrument is stabilized. When collecting the suspended solid water sample, wash the sample bottle for two times with a little of water sample, and then fill the sample bottle to the fully slowly. Ensure the water samples are stored in cool and shady place and transported to the lab. Ensure the water sample be filtered within 24h. The POC samples collected are taken back to the lab; it is better to use the ground glass sample bottle to collect the samples in order to avoid the absorption of C by plastic products. Before use, all the glass containers should be immersed in the Sulfuric acid and potassium dichromate lotion for 24-48h; then be rinsed with tap water and washed again with de-carbonized water, and the de-carbonized water should be prepared in advance. When collection samples in situ, wash the sample bottle with a little of water sample first and then collect the samples and refrigerated transport the sample to the lab for analysis.

10-50 μm viable organisms sample is collected by sediment and concentration counting of certain volume of water sample. There is no need to rinse the sample bottle when collect the samples, collect certain amount of well mixed water samples. Water sample of viable organisms greater than 50μm is prepared by collecting the organisms filtered by a 50μm screen organism net and count the total number. Ensure the organism net is clean and tried before collecting. Viable organism staining agent should be prepared temporarily (to avoid the failure of staining agent as time goes) before sampling. Add quantitative staining agent to the collected viable organism sample for enough time and then add Formalin to fix the sample. Right after the viable organism samples are collected, transport them to the lab (protect them from light and vibration) and analysis as soon as possible.

Personnel conducting the microbe sampling and test should be trained and learn the basic knowledge of microbes. When conduct the microbe testing, all the containers should be sterilized. Prepare a set of trip blank and field blank during each sampling process. One regent blank should be prepared in each batch of samples or ten samples. Keep record of all the original data of the initial dilution water samples for review. Each dilution degree of water sample should replicate.

B6.2 Quality Management of Lab Sample Analysis

B6.2.1 Quality Management of chemical reagents

Chemical reagents used in lab sample analysis should be prepared to solutions in accordance with prescribed conditions. The solutions should be stored in right conditions and used within the prescribed period. The self-prepared solutions are allowed to use unless they are calibrated to be qualified with the guarantee value of national standard solution. The blank value of reagent should be in the same level with the analysis detection limits. If the value is too far over the detection limits, the causes need to be found. And main agents are purified which have great agent blank value or change the reagents (use a new batch number of agents or agents produced by other manufacturers). And all the regents should be checked before use. In the cases when the blank value is hard to be lowered, add appropriate amount of reagent. During analyzing, parallel test the analysis blank and monitor the variation of the blank value.

B6.2.2 Quality management of containers

Make a clear understanding of requirements for the materials used in containers, select the right material. The material of the container should have the least pollution to the water sample and be easy to clean. And it should be inertia to the chemical activity and biological activity to protect the water sample from reacting with the container to the maximum extent. The capacity of dealing with temperature fluctuation, resistance to rupture, sealing property, capacity of reopening, volume, shape, mass and possibility for reuse of the sample storage containers should be taken

consideration when selecting the containers. For most samples which include inorganic compositions, containers which are made of polyethylene, polytetrafluoroethylene or eater polymer are chosen to use; for the storage of samples for determining and analyzing the conductivity and PH in water, containers which are made of high density polyethylene are used; for the storage of organic chemical and organism samples, glass containers are used. The containers should be cleaned in the right way; the compositions of the detergent should not include the substance to be tested. The new container should be cleaned thoroughly; the substances to be tested determine which detergent to choose. For general use, taps water and detergent are used to clean dust and packaging matter, then immersed in the chromic acid and sulfuric acid detergent, and at last rinsed with stilled water. For those used containers, there are usually grease, heavy metal and residents in the bottom and wall of the container, there once they are reused, and they must be cleaned before being used. For those glass containers with stoppers, the ground part is often with digestions and absorptions. Polyethylene is susceptible to absorb oil or grease, heavy metal, sediments and organisms and it is hard to clean. So, much attention should be paid when cleaning the containers made of polyethylene. Before the container made of polyethylene is used, clean with 1 mol/L hydrochloric acid solution and immersed in the (1+3) nitric acid solution for a long time. Before the sample bottle used for storage and environmental parameters analysis is used, clean it with nitric acid solution, and then rinse with stilled water to remove the heavy metal and chromate residual. If the organic composition to be determined is tested after extraction, the glass bottle may be cleaned with extraction detergent.

B6.2.3 Quality management for instrument

The analysis instrument for testing should be in compliance with the stipulations of the Specification for Ocean Survey GB/T12763 and the Specification for Ocean Monitor GB/T17378. Instruments are checked and calibrated by specified personnel regularly. The instruments should be cleaned with stilled water after being used and immersed in the protection liquid to avoid the residual of samples and corrosion of the instruments. Or maintain the instruments according to the instrument operation

manual to keep off of measurement error next time, and conduct the instrument interval check as necessary.

B6.2.4 Quality management for environmental parameters

After the DOC and POC samples are collected, use fiber glass membrane with 47mm diameter and 0.45μm aperture and the standard micropore filter to treat the samples. The membrane must be burned in the 450-500℃ muffle for 24h wrapped in aluminium foil to remove the oxidizing substances (the burning temperature should not exceed 500℃, or else, the filtration characterization of the membrane will change). When the TSS water sample is filtered, clip the membrane with a stainless steel tweezer for fear of pollution. Prevent the seawater from flowing backward and then damage the vacuum pump. And drain out the wastewater in time. Keep the ambient tidy when drying the sample.

Analyze by drawing the standard curve for determination of DOC. Firstly, prepare new chemicals for drawing the standard curve. To assure the quality of the value determined, only if the accuracy of the standard curve reaches to 98% or above, the standard curve is valid.

B6.2.5 Quality management for lab test methods

The lab can undertake the test task on condition that it is accredited the Metrology certificate. Test method is selected mainly based on the precision, accuracy and detection limits of method, to give due consideration of factors such as cost, instrument condition and test cycles and the skill level of personnel. The test methods used should be verified by standard novelty search.

B7. Performance Test, Checkup and Maintenance of Test Instruments

The instrument manager is responsible for compiling the Check List of the Instruments,and establish the file of instruments and identify the instruments with related labels. The operator of instruments should be an authorized staff or one with

vocational test staff certificate. All operators should be approved by the lab to carry out the operation. Use of equipment should be strictly in accordance with operating procedures. The staff is asked to operate the instrument as trained to be in order not to get the invalid test result. The user of the instruments should check the status and environment condition of the instrument (including whether it is in valid period, need maintenance or not, if stabilized or not) before and after use. And fill the Use Record of the Instrument.

If there is abnormal phenomenon (overloading, wrong operation, questionable result displayed) for the equipment, the user should stop the operation and stick a red mark on it. Separate the abnormal instrument to avoid misuse. If the instrument falls out the direct control of the lab for example: removed to other places, sent for repair or calibration, after the instrument is back, the instrument attendant should check the functions and the calibration status of the instrument and recovered to use until the results displayed are satisfying.

The instrument manager takes charge of checking the instruments to prevent the instruments from damaging and losing. Make an inventory of the instruments annually. If there is damage or lose of instrument, repair or handle in accordance with the Control Procedures for Noncompliant Test Work

A specified worker is appointed for the maintenance of the instrument in use. Power on once per month at least (once for 1-2h) to check if the instrument is normal. And these events should be kept record. The instrument manager is responsible for organizing the instrument user to make the routine maintenance plan, and to form the Routine Maintenance Table of Instruments. The instrument user makes the maintenance of the instrument to comply with the items and periodic times in the table and keep the record meanwhile.

B8. Calibration and Frequency of Instruments

Instruments like the spectrophotometer, electronic balance, pH meter,

turbidimeter need to be verified and calibrated by legal metrology verification service agency. The instruments are delivered by the lab synthesizer according to the Quantity Traceability Procedures, aiming to get the qualified certificate. The instrument attendant performs the periodic calibration of the instrument. If the correction factors are obtained after instrument calibration, the instrument attendant is responsible for updating of all the backups and the correcting of related data. The frequency for calibration is once per 12 months, once per 6 months for special instrument.

B9. Data Collection Requirements

The project technical director summarizes the results obtained both from filed work and lab test for shipboard test and organizes the data acquisition and statistics. Prior to data statistics, the test personnel should check the test data first. Check if the original data is integrated and if it meets the requirement, if the calculation and conversion of data is right. Mutual correction is preferred by test personnel after the test data is checked by the test personnel. The reviewer should carry out the review in conformance with the standards, procedures, norms and enforcement rules, and if calculation is required, the calculation formulas and the calculation process should be checked. Check whether the calculations, the rounding off and the conversion are right. The reviewer should review the original data thoroughly at the time of checking the test reports for the reliability and the matching of the data. The data verified to be right is collected and summarized by the technical director.

B10. Data Management

The sampling data and data determined in field should all be record in waterproof table or to create the electronic document right after the samples are taken in field. The management of the electronic data is in accordance with the Procedures for Computer Management.

B10.1 Data Records

To make the record meet the standard and ensure that sufficient information is collected, the lab adopts the form of record table which is uniformed and approved. The sampling records should be prepared with pen or ball-pen. Make sure the record is real, right, complete and clear. There should be date of record, signature of recorded person and the record number on the record, and the technical record should include the signatures of test personnel and reviewers. The technical record should include technical parameters. All the technical parameters, data, observation results and calculations should be kept being recorded in time, with no replenish. If there is any mistake found in the record. Two lines should be written on the original records, while the original record should be made out. Then the modified records should be written on the blank on the top right of the original records with the mender's stamper or signature or abbreviative signature. All the records should be collected, filed and preserved.

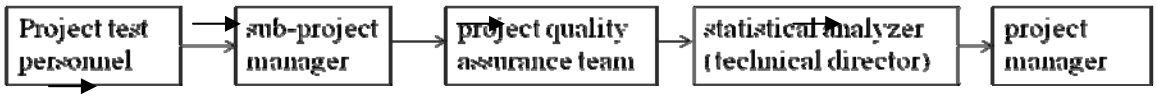
B10.2 Data Confirmation

The test personnel conduct the test in accordance with the requirements and standards related. The quality supervisor takes necessary actions to oversight the test. After the test is finished, the technical director need to sign for confirmation.

B10.3 Data Conversion

The quantity of the plankton is counted and converted to the uniform unit required by the project by statistical analysis. Thereof, organism samples with particle sizes between 10μm and 50μm are converted into cell/ml; particle sizes greater than 50μm are converted to cell/m^3; the bacteria samples are converted to cfu/100ml.

B10.4 Data Delivery



B10.5 Data Analysis

The original data record is collected by related test personnel, and the test personnel will calculate the final test results according to the conversion method of the parameters and the corresponding curve. The calculation process of data is included in the original record. Take the assessment for the uncertainty occurs in the testing process based on the stipulations in the standards for testing all parameter and determine the significant digits for values. At last, the technical director analyzes the removal rate of organisms with different particulate diameters at each steps of ballast water management, and calculates the removal effect. The rounding off method of specific values is as follows:

1) Refer to *The Rule of Data Revising* (GB8170-87) for rounding off the values

The rule for rounding off of the numerical values is a round 5(the digit of the tested valid number is determined): when the rounding off number of the measured value is less or equal to 4, then rounding down; if the rounding off number in the measured value is more than or equal to 6, then rounding up; if the rounding off number in the measured value is equal to 5, rounding up if the mantissa rounded up number is even number, and rounding down if the mantissa rounded up number is odd number. The measured values are rounding off by this way.

In calculating and reading of data, the digits of data might be more than prescribed, for example, the digits of data calculated in calculator may be 7, and when weighed on the analytical balance, only 5 digits of data is obtained, so it is necessary to rounding off the redundant digits. The process for cutting the redundant digits or digit is called the rounding off process, and it is in accordance with the rules of Four Rounding Down and Five Rounding up.

2）Data calculation rules

The data calculation rules are determined by the law of error transmission.

Plus- minus method: transmission of the absolute errors of the measured values. The absolute error of the max absolute error of measured values determines the uncertainty of the analysis result. Therefore, the retention of the significant digit of the summed value of several measured values should base on the number which has the least digits after the decimal point.

Multiply-division method: transmission of the relative errors of the measured values. The relative error of the result should be in accommodation with the value with the max relative error. Therefore, rounding off of the values should be in accordance with the least significant digits.

Scale values of the volumetric containers used for Titrimetric analysis (burette, volumetric flask, pipette) are all with four significant digits. So the number of significant digits of the test data result is four.

3) Formula for calculating removal rate:

$$\text{romoval rate} = \frac{\text{density before treated} - \text{density after treated}}{\text{density before treated}} \times 100\%$$

B10.6 Data Storage and Retrieval

All the test data record should be kept by the data manager. The shelf life of the copies of the original test record, test reports is five years and the data manager takes charge of the safe custody of the files and records. The records should not be let out or loaned to people unrelated and the customer’s business secret should be kept.

If anyone of the internal staff wants to loan or copy of the documents, and he or she should fill in the registration table. For external staff who wants to loan or retrieve the records, he or she should be approved by technical director, after the technical director give approval, he or she can go through the loan procedures and fill in the registration table. Read on site, no taking away. The user or keeper of the records should comply with the procedures for keeping the secret and proprietary of custom,

C. Evaluation and Supervision

C1. Evaluation/supervision and Emergency Response Actions

Project supervisor of the land-based ballast water management system test project is responsible for conducting a continuous improvement actions based on the quality policy, quality objectives, approved result, data analysis, data correction and preventive measures and management review of the lab: analyzing and assessing the status in quo, looking for and finding the aspects needed to be improved (looking for the improvement opportunity); Ensuring improvement aims; establishing improvement scheme and reviewing the scheme, then selecting the best one; Implementing the responsibilities and related resources, and putting forward the improvement scheme; Monitoring and measuring the implementation situation to make sure whether the it is effectively implemented; formally taking the effective measures; the corrective and preventive actions should be taken into the plan and the management of daily improvement activities.

To determine the causes of discrepancies and look for the improvement chance by way of internal approval, management review, custom feedback, ability verification or other way of data analysis of quality control result. If preventive measures are taken, supervise and monitor the implementation of them, to minimize the possibility of nonconformities and look for improvement chance. Conduct the assessment in accordance with the lab' Procedures for Improvement Control, Procedures for Correction Measures, Procedures for Preventive Measures, Procedures for Test Result Quality Control and the Procedures for Management Review Control and take the emergency response measures（see table 12）.

Table 12 Record of the Improvement Actions

Project title		Test parameter	
Test personnel		Test date	
Description of the discrepancies: Recorder: date:			
Causes analysis: Technical director: date:			
Recommendation: supervisor: date:			
The attached record documentation: □_____ □_____ □_____			

C2. Test Report

The technical director of the test organization submits the test reports, and the quality manager of the test organization submits the uncertainty report to the quality management team of land-based test for ballast water management system. The supervisor of the test organization briefly summarizes the results of related

parameters and proposes a new project quality assurance plan to the supervisor of the entrusted organization for summary and renewal.

The testing report of each item and the test results should be precise, clear, objective and conducted in compliance with the test methodology.

Each test reports should include information as follows at least: test designing, identification of the methodology, status description of the tested material and cleared label identification, the acceptance date and the test date, the test result, the test report approver or equivalent mark; if the test result need to be explained, there should be announcement about the test method deviations and evaluation uncertainty included in the test report. In the cases when the testing results provided by a subcontract party are included in the testing report, those results should be marked clearly. The subcontract party should report the results in the way of paper edition or electronic edition.

D. Validity and Usability of Data

D1. Review, Verification and Validation of Data

Check and review all the data from field determination and lab test and verify the integrity, continuity, validity of the data, and check whether the items meet the requirements. Conduct the comparison between the data and the quality objectives set inA7. valid test is indicated when， for both the control tank and ballast water to be treated, with viable organism concentration exceeding 10 times the maximum permitted values in regulation D-2.1 and control tank viable organism concentration exceeding the values of regulation D-2.1 on discharge. When the data results are in consistent with the quality control data of ballast water management system and the data quality achieves the objectives of this project, then the ballast water management system is acceptable.

D2. Verification and Validation Methods

The review, verification and validation of date should be performed to ensure the data meets the criteria. Verify and compare the data with the planned data objectives described in document A7. The verification and validation methods include self assessment, taking part in the reconciliation activities with other labs organized by authorized parties and the ability verification plan. The authorized signatory verifies the quality control data by statistical technology annually and makes the verification reports, then input the management review. If the quality control results are not satisfying or stable, look for the causes for problems and take actions in accordance with the lab's Corrective Measures Procedures, Discrepancy Test Work Control Procedures, and Preventive Measures Procedures. The data validation includes all the task plans of the ballast water management system test except the data verification confirmation, including the quality control result assessment for determination of field sampling data , assessment for determination of lab parameters, discrepancy analysis of sample storage and pretreated, the sample test limitation time range verification, the traceability of methodology for test reagents and test standards, verification of the analysis sensitivity in conformance with QAPP, deviation analysis of sampling and analysis with requirements of QAPP, the verification of calculated results, to ensure that QAPP includes relevant information on all the parameters and samples.

D3. Reconciliation with Test Data Result Objectives

Data generated in this project is analyzed and reconciliated with the data quality and project requirements in accordance with the guidelines for approval of ballast water management systems G8 and discharge requirements of ballast water D-2 regulation. The data meet the requirements of the project and the D-2 standard, and achieve the treatment effectiveness of ballast water management systems and the data related documents will be applied to CCS as appropriate.